Acid-Soluble Deoxynucleotides and DNA Synthesis in Growing Yeast After X-Irradiation, I. Diphenylamine Positive Material in Synchronized and Asynchronously Growing Cells

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In synchronized growing yeast, the level of acid-soluble purine deoxyribonucleotides, as determined by the diphenylamine reaction, fluctuates rhythmically: A rapid increase each time before DNA starts to replicate is followed by a considerable decrease during DNA augmentation. Delay of DNA replication by irradiation of synchronized yeast with 50 kr of X-rays results in a stepwise augmentation of deoxyribose derivatives. The fluctuating behaviour is restored, when DNA starts to increase, again. During asynchronous growth acid-soluble deoxyribosidic compounds are augmented with a considerably increased rate, when DNA replication is delayed by X-rays. The resulting unusual pool seize is maintained, even if DNA replication is resumed to a normal degree, but the rate of further increase is reduced to normal values. Parts of the rapidly accumulated deoxyribose derivatives differ with some properties from purine deoxyribonucleotides.

Irradiation of synchronized yeast with 50 kr of X-rays destroys the proliferation ability of the cells and brings about a considerable delay of DNA replication 1,2. DNA polymerase activity, however, maintains its periodic appearance nearly undisturbed 3. It was supposed, therefore, that repair of radiation-damaged DNA rather than a general lesion of those events, which bring the cell to DNA replication, would cause the delay in DNA increase. In order to learn more details about the mechanisms controlling DNA replication and behaviour of DNA polymerase activity, we were interested in the synthesis of acid-soluble deoxyribonucleotides after X-irradiation. Because of the very small amounts of DNA precursors usually present in growing cells (cf. l.c. 4—7), an optical test system was employed using the diphenylamine reaction for measure of pool seize of acid-soluble deoxynucleotides. Extensive preliminary experiments 10 gave evidence, that nearly exclusively purine deoxyribose derivatives accounted for the reaction with yeast extracts. Some of the results presented here were reported in a preliminary form 8,9.

Experimental

Baker's yeast (Saccharomyces cerevisiae, "Germania-Hefe", Norddeutsche Hefewerke GmbH, Hamburg) was synchronized as described previously 2. Asynchronously dividing yeast was grown from a cell clone derived from baker's yeast on nutrient agar 10. Growth media, conditions of culture, irradiation with X-rays and related details are described earlier 1, 5, 9.

** Extraction of acid-soluble cell constituents **

The cells from 200 ml culture (1010 cells at "zero time" of each experiment) were collected by centrifugation (2 °C), washed with 10 ml of icewell water, and extracted with 3,5 ml TCA ** for 30 min. at 25 °C. The insoluble material was centrifuged off, and in the clear supernatant ("cell extract") the acid-soluble "deoxyribosides" ** were estimated. The residue was analyzed for DNA and protein content according to l.c. 3.


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** Abbreviations: TCA = trichloroacetic acid, O.D. = optical density, "deoxyribosides" means the acid-soluble diphenylamine positive cell constituents, turned out as mainly deoxyribose derivatives.
Estimation of acid-soluble diphenylamine positive compounds

3.0 ml cell extract were heated with 6.0 ml diphenylamine reagent\textsuperscript{11} in a boiling water bath (10 min.). The samples were cooled in an ice bath (5 min.), and O.D. was measured at 600 nm (4 cm cells). The data were calculated for nmoles “deoxyribosides” per 200 ml culture from a deoxyribose standard. The color intensity (O.D.) was proportional to the number of cells extracted, in the range between at least 0.5 \times 10^9 and 5.0 \times 10^9 cells. Preliminary experiments\textsuperscript{10}, including behaviour against activated charcoal (prepared according to 12), adsorbability and elution pattern with the anion exchange resin Dowex 1 x 10 (formate form, cf. 14), and with Sephadex G-15, resp., and susceptibility to hot dilute acid, gave evidence, that more than 90\% of the acid-soluble yeast constituents showing a diphenylamine reaction under the above test conditions are deoxyribonucleotides. From standard solutions of numerous substances only deoxyribose and purine deoxynucleotides exhibited a remarkable colour\textsuperscript{10}.

Results

The behaviour of the acid-soluble diphenylamine positive substances (“deoxyribosides”) during a cell cycle

In extracts from synchronized growing yeast the amount of diphenylamine positive material (“deoxyribosides”) fluctuates rhythmically during a cell cycle (Fig. 1 B). Maximum values are found shortly before the DNA content of the cells begins to rise. During “S-phase” the level is diminished drastically. It increases, again, at the end of DNA synthesis, and the pool is replenished in the period before starting the next DNA replication. From each generation to the following one, the “deoxyribosides” maximum is increased by a factor of two corresponding to the rhythmic doubling of cell number and DNA content in the synchronized growing cultures.

The effect of X-irradiation

The oscillating behaviour of “deoxyribosides” is altered to a stepwise increase, when DNA synthesis is delayed by irradiation of synchronized yeast with 50 kr of X-rays (Fig. 1 C). The timing of the augmentation remains nearly unaffected, the values are doubled in a synchronized manner with each generation time. Only the periodic decrease of “deoxyribosides” is omitted nearly completely, as long as DNA synthesis is delayed. At the same time, however, when DNA synthesis is resumed, the pool of “deoxyribosides” is diminished, again, resulting in a recovery of fluctuating behaviour of “deoxyribosides” consistent with the normalization of DNA replication.

In X-irradiated asynchronously growing cells the rate by which deoxyribosidic compounds are increased is enhanced considerably, as long as DNA synthesis is delayed (Fig. 2). Thus the values exceed the control just after a short time. The rate of the increase is “normalized” but in the same way, as the synthesis of DNA is resumed to a normal degree. The pool maintains its unusual height, however, for at least three hours of further cell growth.

About 23\% of the acid-soluble diphenylamine positive material from X-irradiated yeast are not adsorbed by charcoal, when the samples are taken at

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Fig. 2. Acid-soluble "deoxyribosides" and DNA increase in X-irradiated asynchronously growing yeast. Samples (0–200 min.: 200 ml; 210–300 min.: 100 ml) were taken from asynchronously growing cultures (log phase) at the indicated times, and cell constituents were estimated as described in "Experimental". All data are related to 200 ml culture (10^8 cells at "zero time").

<table>
<thead>
<tr>
<th>Samples taken at time [min.]</th>
<th>Culture</th>
<th>Remaining in solution [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>&quot;deoxyribosides&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>untreated</td>
</tr>
<tr>
<td>0</td>
<td>control</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>irradiated</td>
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<tr>
<td>180</td>
<td>control</td>
<td>90.7</td>
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<tr>
<td></td>
<td>irradiated</td>
<td>134.4</td>
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</tbody>
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Tab. 1. Adsorption of acid-soluble deoxyribosidic compounds from X-irradiated yeast by activated charcoal. 200 ml samples from asynchronously growing cultures were taken at the indicated times, and cell extracts were prepared. The cell extracts were treated with Norit A (Serva, Heidelberg), which was activated according to I.e.\(^\text{12}\), in the following way: 25 mg Norit were added to 3.5 ml extract, and the samples were left for 60 min. at 0 °C with occasional gentle shaking. Then 0.2 ml ethanol were layered on the suspension (cf. 13), and Norit was centrifuged off (15 min., 0 °C, 10,000 r.p.m.). Aliquots from the clear supernatants (aqueous phase) were taken for the diphenylamine assay. O.D. was calculated as nmoles "deoxyribosides" per 200 ml culture (10^8 cells at "zero time") from a deoxyribose standard. For further details see "Experimental".

"zero time" of incubation (Tab. 1). These non-adsorbable substances are increased by about 40% during the first 180 min. of cell growth. The total of acid-soluble "deoxyribosides" is augmented, however, by about 190% in this time, and it exceeds the control considerably (50%). Thus the accumulation of "deoxyribosides" mainly concerns deoxyribonucleotides (and precursors), though some of the diphenylamine positive substances from X-irradiated yeast obviously represent other material.

Inhibition of protein synthesis by cycloheximide at a dose (5 × 10^{-7} M) just destroying the proliferation ability of unirradiated cells, affects the behaviour of "deoxyribosides" in different ways: Only a slight effect on the first increase is seen both with X-irradiated and unirradiated control cells, when the inhibitor is added at "zero time" of incubation. As shown in Tab. 2, even after 360 min. the values from X-irradiated yeast exceed the control, though at this time the augmentation of diphenylamine positive material apparently is damaged by cycloheximide in general. When cycloheximide is added after 80 min. of incubation, however, that is at a time just beginning DNA synthesis in X-irradiated yeast (cf. Fig. 1), a pronounced inhibitory effect on the further increase of "deoxyribosides" is seen particularly with the irradiated cells, whereas in the control the augmentation is prevented only to a minor degree.

**Discussion**

Estimation of acid-soluble deoxyribosidic compounds (DNA precursors) in cell extracts requires


methods sensitive and specific enough for the low level of these cell constituents (cf. 1. c. 4-7). Single components of the deoxynucleotide pool were studied on the basis of chromatographic analysis 4, 5, 7, 14, 16, but these methods hardly appear applicable on series of samples from rapidly growing cell systems. Our resolution to study the pool of acid-soluble deoxyribose derivatives from growing yeast by means of the relatively unspecific DISCHE reaction, was based on extensive preliminary experiments 10 showing, that unspecific side reactions (cf. 1. c. 11-21) do not influence the determination of deoxyribose derivatives in yeast extracts to a remarkable degree, and giving evidence, that almost all of the diphenylamine positive cell constituents represent purine deoxynucleotides.

In extracts from synchronized growing yeast rhythmic fluctuations of the "deoxyribosides" are seen, which suggest an interaction between periodic supply with DNA precursors and DNA replication in the growing cell. A limited (periodical) synthesis of DNA precursors during a cell cycle should be expected from regulation by induction and feedback mechanisms (cf. 1. c. 22), and is emphasized also by fluctuating activities of deoxynucleotide synthetizing enzymes (cf. 1. c. 23-27). Our findings, that in X-irradiated synchronized yeast the level of "deoxyribosides" decreases only little, as long as DNA replication is delayed, but that it is reduced immediately when DNA augmentation is resumed, strongly support an interpretation of the pool oscillations in yeast on the basis of limited deoxynucleotide synthesis and incorporation into DNA.

With asynchronously growing yeast, an accumulation of deoxyribosidic compounds is found after X-irradiation. As shown by other authors, deoxyribose derivatives are increased also in bacteria and animal cell systems after application of certain cytostatic drugs, and of X-rays, resp. 4-6, 28-34. The kinetics of the accumulation in X-irradiated yeast exhibit clear dependence on delay in DNA synthesis, by normalizing the rate of increase as soon as DNA synthesis is resumed. As derived from synchronized growing yeast, this behaviour of "deoxyribosides" results from a deficient decrease during the delay of DNA synthesis, whereas the rhythmic increase remains nearly unaffected. The accumulation depends largely on a preceding synthesis of protein.

Nevertheless it is not clear, if the material accumulated really are DNA precursors. A rapid degradation of DNA into acid-soluble oligodeoxynucleotides after X-irradiation is described from Escherichia coli 15 T-35, but an accumulation of deoxynucleoside triphosphates by de novo synthesis is seen only when the feedback control by dTTP is abolished 7. Gel filtration of yeast extracts on Sephadex G-15 or G-10 shows no increase of UV-absorbing material in the void volume of the column after X-irradiation of the cells 19. On the other hand X-irradiation brings about a rapid formation of deoxyribosidic compounds distinct from deoxynucleo-

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tides. Origin and nature of these substances, which are characterized by their lacking binding capacity for charcoal, are not yet clear. CHOU and SCHERBAUM working with Tetrahymena described an accumulation of acid-soluble phosphorylated deoxysugars by release from existing structures after a heat treatment. The question, if degradation products from DNA (e.g. deoxynucleosides, cf. l.c.32, 33) are formed also in yeast after X-irradiation, needs further investigation.

An interpretation of the nearly undisturbed increase of “deoxyribosides” after X-irradiation by mechanisms other than periodical deoxynucleotide synthesis, e.g. by partial DNA degradation and repair, seems difficult, however, for it would imply similar periodicity and similar timing of the basic events.

Taken together our data indicate, that the rhythmic increase of “deoxyribosides” in synchronized growing yeast is regulated independently from DNA replication. On the other hand, the intracellular level of “deoxyribosides” apparently does not trigger the rate of DNA synthesis.

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On the Biosynthesis of Juvenile Hormone in the Adult Cecropia Moth

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Juvenile Hormone, Biosynthesis, Cecropia Moth

In adult males of the giant silk moth Hyalophora cecropia (L.) the amount of juvenile hormone (JH) extractable from the abdomens increases sharply between the first and fourth day of adult life: 4—8 day-old moths contain up to 6 µg. During the biosynthesis, L-methionine provides the ester methyl group of both JH and its lower homologue JH-II. It does not contribute to the formation of the carbon skeleton. Farnesol, farnesyl pyrophosphate, and propionate are not utilized. Mevalonate is extensively incorporated into trans,trans-farnesol, but not into the sesquiterpene-like hormone. This result indicates that JH is not synthesized via mevalonate in the adult moths. Label of 2-14C-acetate was recovered in both JH and farnesol; the incorporation rate, however, was very small. The label of JH was located in the carbon skeleton.

The juvenile hormone1, 2 (JH; 1) is one of the major endocrine components which regulate post-embryonic growth and development in insects. This hormone and its lower homologue 3 (JH—II; 2) seem to be closely related biochemically to acyclic sesquiterpenes like farnesol (3). Most remarkable, however, are the ethyl groups at positions C-7 and C-11. This type of substitution is without precedence among natural isoprenoid compounds. Ethyl side chains have been found in some phytosterols 4 but in no case do they replace a methyl group bio-

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